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Assignment of the μ_4-O5 atom in catalytic center for water oxidation in photosystem II

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The detailed structure of catalytic center of water oxidation, Mn_4Ca-cluster, in photosystem II (PSII) has been reported recently. However, due to the radiation damage induced by X-ray and the complexity of the Mn_4Ca-cluster, the assignment of the μ_4-O5 atom coordinated by three Mn and one Ca^{2+} ions is still lack of essential evidences. In this article, we synthesized one Mn complex containing two μ_4-O atoms. It is found that the lengths of all μ_4-O–Mn bonds in this Mn complex are in the range of 1.89–2.10 Å, which are significantly shorter than 2.40–2.61 Å distance of μ_4-O5–Mn bonds in Mn_4Ca-cluster observed in the crystal structure of PSII. In addition, DFT calculations have been carried out on the Mn_4Ca-cluster. It is found that the O atom of μ_4-O or μ_4-OH always trends to deviate from the center position of four metal ions, resulting in unequal bond lengths of four μ_4-O–M (M=Mn or Ca), which is obviously different with larger and nearly equal distances between μ_4-O and four metal ions observed in the crystal structure. Based on these results, we suggest that the μ_4-atom in Mn_4Ca-cluster of PSII is unlikely to be a μ_4-O, μ_4-OH or μ_4-OH_2, and its assignment is still an open question.

photosystem II, Mn-cluster, water oxidation, Mn-complex, DFT calculation

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Mn_4Ca-cluster is the catalytic center for water oxidation in photosystem II (PSII) [1,2]. The turnover of the Mn_4Ca-cluster leading to water oxidation involves five different states (S*n*, *n*=0–4), wherein S_0 state is the initial state and S_1 state is a dark stable state. The structure of Mn_4Ca-cluster and the mechanism of water oxidation are the most important topics in the field [1,3–6]. Extensive investigations have been reported in literatures.

Recently, Umena et al. [7] have reported the crystal structure of PSII at a resolution of 1.9 Å, which revealed the detailed structure of the Mn_4Ca-cluster. The core of the Mn_4Ca-cluster is shown in Figure 1, wherein four Mn and one Ca ions are connected by five μ-O atoms. The distances between μ_3-O/μ_2-O and Mn ions are all in the range of 1.8–2.1 Å; while the lengths of three μ_4-O5–Mn bonds are in the range of 2.4–2.61 Å. The latter is significant longer than those of μ_2-O–Mn or μ_3-O–Mn bonds. The apparently larger and nearly equal lengths of three μ_4-O–Mn bonds indicate that the μ_4-O5 atom is very special. In fact, this μ_4-O5 atom has attracted extensive attentions by theoretical studies recently, and several groups [8–10] suggested that the μ_4-O5 atom may play crucial roles to provide one oxygen source for the O–O bond formation. However, due to radiation damage induced by X-ray [11,12] and the complexity of the Mn_4Ca-cluster, especially the assignment of this μ_4-O5 atom was suffered by its weak electron density compared with all other O atoms in Mn_4Ca-cluster in X-ray diffraction data [7]. Therefore, the assignment of μ_4-O5 is still an open question. Here, we have carried out DFT calculations on Mn_4Ca-cluster to check the rationality of the assignment of μ_4-O5 atom. In addition, we have also synthesized a manganese complex containing μ_4-O atom. The structural analysis on this compound further provides a clue to demonstrate the possible structure characters of the μ_4-O in

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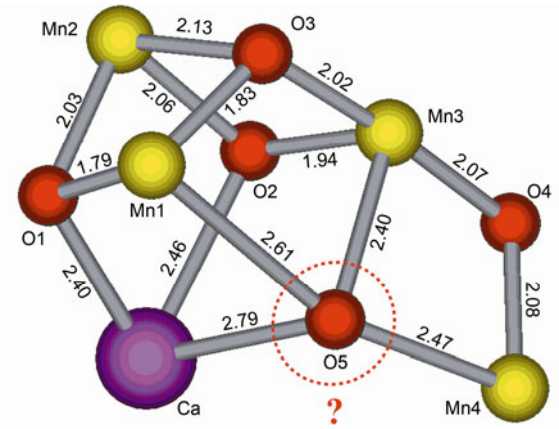

Figure 1 Core of Mn_4Ca-cluster in PSII. O, Mn and Ca are shown in red, yellow and violet, respectively. The values are bond lengths (Å). The μ_4-O5 atom is marked in red dashed circle.

Mn_4Ca-cluster as suggested in the crystal structure of PSII.

1 Experiment and calculations

1.1 Synthesis of Mn complex

To a solution of the tetrabutyl ammonium salt of catecholate dianion $[C_6H_4O_2(NBu_4)_2]$ (1.6 mmol) in acetonitrile and pyridine (v/v=10∶1), the tetrabutyl ammounium salt of 2,2-dimethy propionate anion $[C_5H_9O_2(NBu_4)]$ (1.2 mmol), $La(NO_3)_3$ (0.4 mmol) and $MnBr_2$ (2.0 mmol) were added in sequence. The solution was stirred at room temperature overnight. The crystal was formed after leaving the solution at room temperature for a few days. The single crystal with dimension of 0.20 mm×0.15 mm×0.12 mm was used to collect the X-ray diffraction data. Crystal data: orthorhombic space group *Pncb*, a=13.948(3) Å, b=23.071(5) Å, c=27.758(6) Å, α=90.0, β=90.0, γ=90.0, Z=4. The detail structure is shown in Figure 2.

1.2 DFT calculations

The initial structure model for S_0 state of the Mn_4Ca-cluster was constructed on the basis of 1.9 Å resolution X-ray structure [7]. The ligations of D_1-Asp_{170}, D_1-Glu_{189}, D_1-Glu_{333}, D_1-Asp_{342}, D_1-Ala_{344}, CP_{43}-Glu_{354} and D_1-His_{332} to Mn_4Ca-cluster were simplified by six $CH_3CO_2^-$ and one imidazole, respectively. According to the previous electron paramagnanetic resonance (EPR) [13,14] and extended X-ray absorption fine structure (EXAFS) [15,16] studies, the valences of four Mn ions are S_1 (+4, +4, +3, +3), S_0 (+4, +3, +3, +3) or (+4, +4, +3, +2). Considering the Mn_4Ca-cluster is located inside of proteins, DFT calculations were only performed on the model containing 0, or +1 or −1 net charge. For simplify, all atoms except the μ_4-bridged atom and the active hydrogen atoms of H_2O or OH groups were frozen during the structure optimization. High spin states were applied to four Mn ions in all calculation models. DFT calculations were carried out by using the hybrid functional B3LYP at the Lanl2dz basis. All DFT calculations were carried out by using the Gaussian03 program [17].

2 Results and discussions

From Figure 2, one can clearly see that the peripheral ligands are provided by ten RCO_2^- groups and four pyridines, forming a hydrophobic environment of Mn_6O_2 core. The whole charge of the cluster in Figure 2 is zero. In core of this compound, four Mn^{2+} ions and two Mn^{3+} ions are present. The distance between Mn ions are 2.8 Å (Mn^{3+}–Mn^{3+}), 3.5 Å (Mn^{2+}–Mn^{3+}) and 3.7 Å (Mn^{2+}–Mn^{2+}), respectively. Interestingly, there are two μ_4-O atoms which are similar to the μ_4-O5 of the Mn_4Ca-cluster in PSII. The only difference is that the μ_4-O5 atom is coordinated by three Mn ions and one Ca in PSII; while it is coordinated by four Mn ions in synthesized Mn complex. Therefore, we believe that the structural analysis on the compound in Figure 2 will provide clues to understand the uncertainty of the structure of the Mn_4Ca-cluster in PSII. It is found that the μ_4-O atom in the Mn complex is nearly located in the center position of four Mn ions, and the lengths of four μ_4-O–Mn bonds are 2.19,

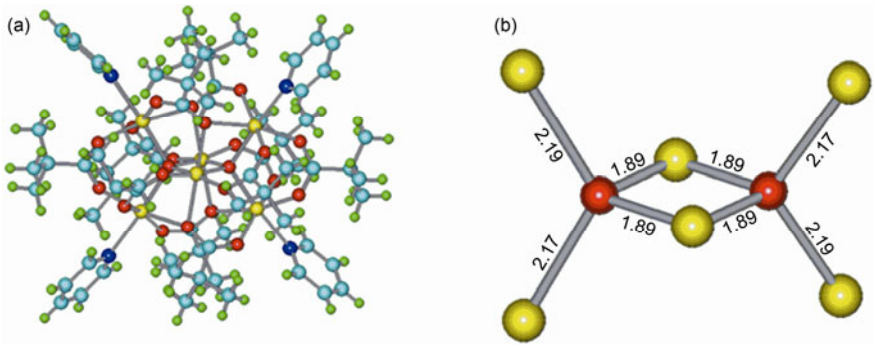

Figure 2 The whole (a) and core (b) structure of the Mn complex. H, O, N, C and Mn are shown in green, red, blue, cyan and yellow, respectively. The values present the bond lengths (Å).

2.17, 1.89 and 1.89 Å, respectively. The average length of all μ_4-O-Mn bonds is 2.04 Å, and the difference between the shortest and the longest bonds is only 0.3 Å. As we know, in Mn_4Ca-cluster (see Figure 1), the average length of three μ_4-O5-Mn bonds is 2.49 Å, and the difference between the shortest bond and the longest bond is 0.21 Å [7]. Therefore, it means that the average length of μ_4-O5–Mn bonds in the Mn_4Ca-cluster is 0.45 Å longer than that in Mn complex in Figure 2. It is noticed that the μ_4-O atom only interacts with four metal ions, and no further interaction with surroundings in both synthesized Mn-complex and Mn_4Ca-cluster in PSII. Considering this similar coordination environment and net charge of the Mn-complex in Figure 2 and the Mn_4Ca-cluster in PSII, we doubt that such a big difference related with μ_4-O atom in two systems is reasonable. On the contrary, we suggest that the assignment of μ_4-O5 atom in the crystal structure of PSII would be unreliable.

To further explore the rationality of the μ_4-O5 in Mn_4Ca-cluster, theoretical calculations were carried out. Theoretical calculations have been widely used to study the structure and mechanism of various enzymes including PSII [18,19]. We have reported theoretical studies on the secondary electron donor [20,21] and Mn_4Ca-cluster [22] and succeeded to predict the Ca ion site in Mn_4Ca-cluster. Here we perform DFT calculations on Mn_4Ca-cluster to check the rationality of the assignment of μ_4-O5 atom in X-ray crystal structure.

Calculation models of the Mn_4Ca-cluster in S_0 and S_1 states are shown in Figure 3. In S_0 state, the lengths of three μ_4-O–Mn bonds are 2.99, 1.87 and 1.99 Å, respectively. The difference between the shortest and the longest bonds is 1.12 Å. In S_1 state, the lengths of μ_4-O–Mn bonds are 2.95, 1.82 and 2.03 Å, respectively. The difference between the shortest and the longest bonds is 1.13 Å. In both models the distance between μ_4-O atom and Ca ions is about 2.50 Å. Obviously, the μ_4-O atom in the two models in Figure 3 is deviated from the center position of the four metal ions, resulting in the unequal distances between μ_4-O atom and four metal ions.

Considering the lower electron density of the μ_4-O5 atom in X-ray diffraction data, Umena et al. [7] proposed that μ_4-O5 might be a μ_4-OH. Therefore, we also constructed various models containing μ_4-OH as shown in Figure 4. Under this condition, in S_0 state, the lengths of μ_4-O-Mn bonds are 2.88, 2.22, and 2.14 Å, respectively. In S_1 state, the lengths of μ_4-O–Mn bonds are 2.85, 1.99, and 2.13 Å, respectively. Obviously, the O atom of the μ_4-OH group in these two models is deviated from the center position as well. Again they are significantly different with the position of μ_4-O5 atom in the Mn_4Ca-cluster observed in X-ray structure data.

It is noticed, Gatt et al. [23] suggested that the μ_4-O5 atom of the Mn_4Ca-cluster might be a μ_4-OH_2, and they

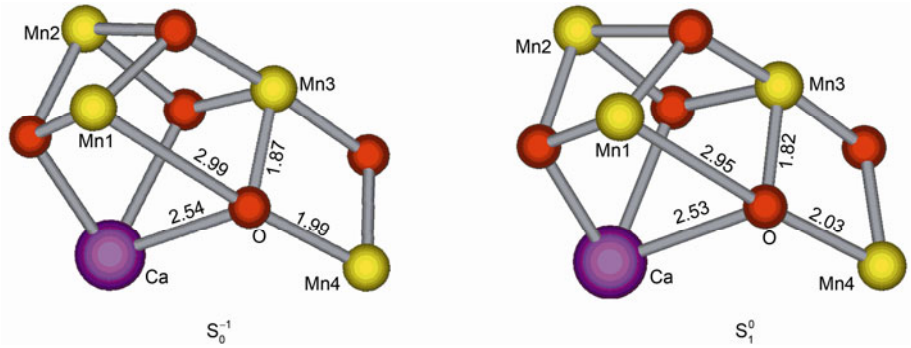

Figure 3 Calculation models of the Mn_4Ca-cluster. The μ_4-atom is μ_4-O^{2-}. All other illustrations are the same as those in Figure 2.

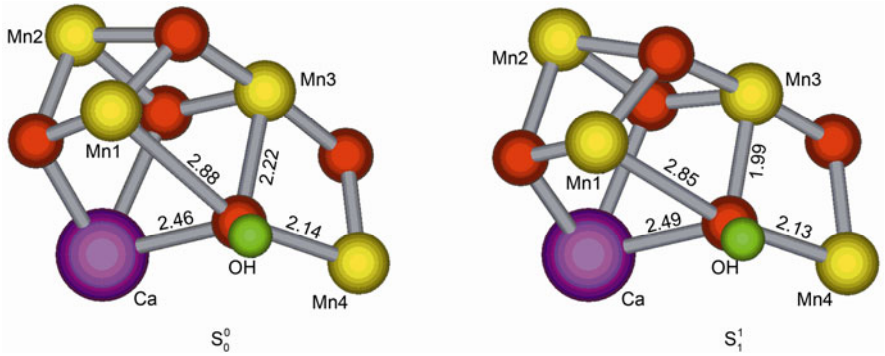

Figure 4 Calculation models of the Mn_4Ca-cluster. The μ_4-atom is μ_4-OH^-. All other illustrations are the same as those in Figure 2.

concluded that the four Mn ions valences are S_0 (+3, +3, +3, +2) and S_1 (+3, +3, +3, +3), respectively. However, these valences of four Mn ions are obviously controversial with the widely accepted valences of S_1 (+4, +4, +3, +3) and S_0 (+4, +3, +3, +3) or (+4, +4, +3, +2) observed by EPR [13,14] and EXAFS [15,16].

Our DFT calculations clearly show that the μ_4-O atom is always deviated from the center position of the four metal ions resulting in unequal distances between μ_4-O atom and three Mn ions, which is obviously different with the central position observed in crystal structure of PSII. These results further indicate that the μ_4-atom of the Mn_4Ca-cluster in the crystal structure of PSII might be something else, instead of μ_4-O, μ_4-OH or μ_4-OH_2.

3 Conclusions

In summary, both our DFT calculations on the Mn_4Ca-cluster and the structural analysis on the synthesized Mn-complex containing μ_4-O atom indicate that the assignment of μ_4-O5 in the Mn_4Ca-cluster in crystal structure would be unreliable, and also doubt the various mechanism models for water oxidation proposed recently, wherein the μ_4-atom was suggested to act as an oxygen source to form O–O bond [8,10]. It is suggested that the μ_4-atom of Mn_4Ca-cluster in PSII is unlikely to be O, OH or H_2O, and the clear assignment of this atom needs to be further studied in future. Our group is currenty working on this subject.

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